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REVIEW ARTICLE

Histone Deacetylase Inhibitors in Cancer Therapy

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See accompanying article on page 5410

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ABSTRACT

Purpose
Epigenetic processes are implicated in cancer causation and progression. The acetylation status of histones regulates access of transcription factors to DNA and influences levels of gene expression. Histone deacetylase (HDAC) activity diminishes acetylation of histones, causing compaction of the DNA/histone complex. This compaction blocks gene transcription and inhibits differentiation, providing a rationale for developing HDAC inhibitors.

Methods
In this review, we explore the biology of the HDAC enzymes, summarize the pharmacologic properties of HDAC inhibitors, and examine results of selected clinical trials. We consider the potential of these inhibitors in combination therapy with targeted drugs and with cytotoxic chemotherapy.

Results
HDAC inhibitors promote growth arrest, differentiation, and apoptosis of tumor cells, with minimal effects on normal tissue. In addition to decompaction of the histone/DNA complex, HDAC inhibition also affects acetylation status and function of nonhistone proteins. HDAC inhibitors have demonstrated antitumor activity in clinical trials, and one drug of this class, vorinostat, is US Food and Drug Administration approved for the treatment of cutaneous T-cell lymphoma. Other inhibitors in advanced stages of clinical development, including depsipeptide and MGCD0103, differ from vorinostat in structure and isoenzyme specificity, and have shown activity against lymphoma, leukemia, and solid tumors. Promising preclinical activity in combination with cytotoxics, inhibitors of heat shock protein 90, and inhibitors of proteasome function have led to combination therapy trials.

Conclusion
HDAC inhibitors are an important emerging therapy with single-agent activity against multiple cancers, and have significant potential in combination use.

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INTRODUCTION

Epigenetic modulation of gene expression is as an important regulatory process in cell biology.[1] Gene regulation occurs in the context of packaging of DNA into an organizing structure, the nucleosome, composed of a DNA strand wound around a core of eight histone proteins.[2] The N-terminal tails of each histone extend outward through the DNA strand. Amino acid residues on the histone tail are modified by post-translational acetylation, methylation, and phosphorylation.[3] These modifications change the secondary structure of the histone protein tails in relation to the DNA strands, increase the distance between DNA and histones, and increase accessibility of transcription factors to gene promoter regions.[4] Deacetylation, demethylation, and dephosphorylation of histones have the opposite effect of decreasing access of transcription factors to promoter regions. Developmental and regulatory processes within the cell are strongly influenced by histone modification. Emerging data now implicate histone modification in the pathobiology of cancer and other diseases. Histone acetylation is mediated by histone acetyl transferases,[5] while acetyl groups are removed by histone deacetylases (HDACs).[6] This review will focus on the current role and potential of HDAC inhibitors in cancer treatment. Histone methylation[7] and phosphorylation,[8] also the subjects of therapeutic research, are less well understood processes, and will not be considered in this discussion.

The HDAC inhibitor vorinostat (suberoylanilide hydroxamic acid) is approved for treating refractory cutaneous T-cell lymphoma (CTCL).[9] Other HDAC inhibitors have entered clinical trials in both solid tumors and hematologic malignancies. Herein we summarize the biology of HDAC proteins and their postulated role in cancer pathogenesis. We describe the HDAC inhibitors under

clinical investigation, their pharmacologic properties, their approved indications, and their potential as single agents and in combination therapy.

BIOLOGY OF HDACs

In the 1970s, the Friend erythroleukemia cell line was found to differentiate in the presence of dimethyl sulfoxide or butyrate.[10,11] Many compounds with the ability to promote differentiation of tumor cell lines, particularly those with a planar-polar configuration, induced accumulation of hyperacetylated histones.[12] Histone hypoacetylation was also found to be associated with gene silencing, as in the inactivated female X chromosome.[13] Seminal experiments showed that treatment of cells with the short-chain fatty acid sodium butyrate caused hyperacetylation of histone octamers. This histone modification increased the spatial separation of DNA from histone and enhanced binding of transcription factor complexes to DNA.[14] Later, the first mammalian HDACs were cloned on the basis of their binding to known small molecule inhibitors of histone deacetylation.[15] These genes were homologous to yeast transcriptional repressors, strengthening the evidence that histone deacetylation suppresses gene expression.

Further work has identified at least 18 human HDACs, with varying function, localization, and substrates (Table 1).[16,17] The four classes of HDACs are grouped by their homology to yeast proteins. Classes I, II, and IV all contain a zinc (Zn) molecule in their active site and are inhibited by the pan-HDAC inhibitors. The seven different class III HDACs (sirtuins), are homologous to the yeast Sir2, do not contain Zn in the active site, and are not inhibited by any current HDAC inihibitors.[18]

The function of many nonhistone proteins is also controlled by acetylation on lysine residues, and, in fact, HDACs may have appeared evolutionarily before histone-like genes.[19,20] HDAC-mediated deacetylation alters the transcriptional activity of nuclear transcription factors, including p53,[21] E2F,[22] c-Myc,[23] nuclear factor κB (NF-κB),[24] hypoxia-inducible factor 1α (HIF-1α),[25]as well as estrogen receptor α[26] and the androgen receptor complexes.[27] Other cancer-related proteins are acetylated, including the DNA repair enzyme Ku70,[28] the chaperone heat shock protein 90 (HSP90),[29] the signaling pathway intermediate STAT3,[30] and alpha-tubulin.[31]

Knockout mouse models have demonstrated the importance of HDACs in cell differentiation. Genetic deletion of the class I genes HDAC1[32] or HDAC2[33] results in embryonic or perinatal lethality, respectively. Class II HDAC knockout mice are viable and fertile (with the exception of HDAC7 knockouts), but all display developmental abnormalities.[34] HDAC inhibitors recapitulate these findings. In many tumor cell lines, inhibition or down-regulation of HDACs causes the upregulation of the cell cycle gene $p21^{WAF1/CIP1}$, blocking the Cyclin D/CDK4 complex, and leading to cell cycle arrest and differentiation.[35,36]

HDACs also have a critical role in modulating the balance between pro- and antiapoptotic proteins.[37] HDAC inhibition upregulates the intrinsic apoptosis pathway through induction of the proapoptotic genes *Bmf*[38] and *Bim*.[39] Apoptosis induced by HDAC inhibitors can be blocked by overexpression of antiapoptotic Bcl-2.[40] Further, hyperacetylation stabilizes the p53 protein, promoting both cell cycle arrest and expression of proapoptotic genes.[41] HDAC inhibition also induces elements of the extrinsic apoptotic pathway by increasing the expression of death receptor proteins, including Fas, TNF-α, and the TRAIL receptor.[42,43]

HDAC inhibition may also affect tumor cell survival by blocking tumor angiogenesis, and by inhibiting intracellular stress response pathways. HDAC inhibitors increase acetylation of the pro-angiogenic transcription factor HIF-1α, enhancing its degradation.[25] In addition, HDAC inhibitors decrease expression of the vascular endothelial growth factor receptor.[44] HDAC inhibition also increases generation of intracellular reactive oxygen species, and impairs handling of misfolded proteins by influencing endoplasmic reticulum stress responses.[45,46] By inducing hyperacetylation of HSP90, HDAC inhibitors disrupt the function of this critical protein chaperone that normally protects cellular and cancer-related proteins from degradation (Fig 1).[29] Affected oncogenic proteins include BCR-ABL, epidermal growth factor receptor, human epidermal growth factor receptor 2/*neu*, FLT3, Akt, and c-Raf.[17,47] The important effects of HDAC inhibition on HSP90 function have stimulated interest in clinical trials of the combination of HDAC and HSP90 inhibitors.

As summarized earlier, modulation of histone and, more generally, protein acetylation, alters pathways that promote proliferation, angiogenesis, and survival in cancer cells. Moreover, HDAC inhibitors have global effects on gene expression, and may affect as yet unrealized cellular processes.[48-50] Successful therapeutic use of HDAC inhibitors may thus depend on subtleties of the cellular milieu, the specific HDACs targeted, and the relative dependence of the malignant phenotype on the unique set of pathways influenced by a specific drug.

HDACs IN CANCER

A common finding in cancer cells is high level expression of HDAC isoenzymes and a corresponding hypoacetylation of histones.[51,52] A study of normal and malignant tissues revealed a consistent pattern: higher levels of histone acetylation in normal lymphoid tissue as compared to lymphomas, and in normal colonic epithelium as compared

Table 1. HDACs

Class	Enzymes	Zn^{2+} Dependent	Localization	Expression
I	HDAC1, HDAC2, HDAC3, HDAC8	Yes	Nucleus	Ubiquitous
IIa	HDAC4, HDAC5, HDAC7, HDAC9	Yes	Nucleus and cytoplasm	Tissue specific
IIb	HDAC6, HDAC10	Yes	Cytoplasm	Tissue specific
III	Sirtuins 1-7	No	Variable	Variable
IV	HDAC11	Yes	Nucleus and cytoplasm	Ubiquitous

Abbreviation: HDAC, histone deacetylase; Zn, zinc.

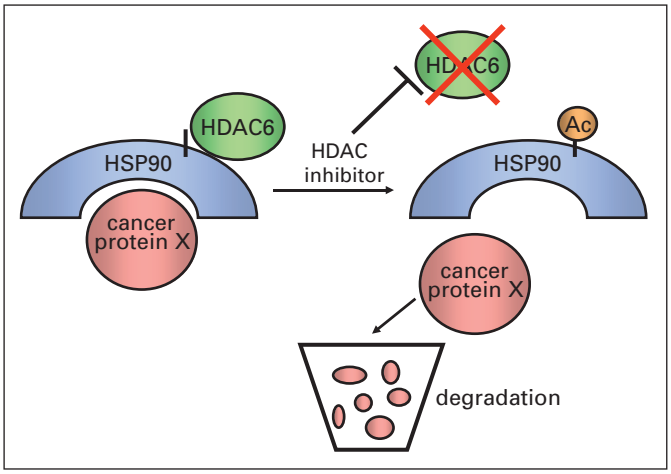

Fig 1. The interaction between heat shock protein 90 (HSP90) and histone deacetylase 6 (HDAC6). HSP90 chaperones several oncogenes (cancer protein X). HSP90 function is inhibited by acetylation on lysine residues. When cells are treated with an HDAC inhibitor, HDAC6 activity is blocked, HSP90 becomes hyperacetylated (Ac), and client cancer proteins are degraded by the proteasome.

to colon adenocarcinomas.[53] HDAC1 enzyme expression is higher in colon adenocarcinoma cells than in normal colon epithelium.[54] Loss of genetic gatekeeper function in precancerous lesions may be associated with increased activity of HDACs. HDAC2 expression is elevated in colon cancer cells, possibly as a result of adenomatosis polyposis coli (*APC*) gene deficiency, an early event in colon carcinogenesis. Knockdown of HDAC2 expression by short inhibitory RNAs or treatment with an HDAC inhibitor–induced growth arrest of a colon cancer cell line, and HDAC inhibitor-treated APC mutant mice developed fewer colon adenomas than untreated animals.[55]

Increased HDAC activity, and the resultant transcriptional repression of genes essential to hematopoietic differentiation, may play a critical role in the pathogenesis of certain leukemias. The PML-RARα protein product of the t(15;17) translocation in acute promyelocytic leukemia, as well as the core binding factor leukemia gene products AML1-ETO and CBFβ-MYH11 act as transcriptional repressors through their recruitment of HDACs to gene promoter regions. All-*trans*-retinoic acid, an effective therapy for acute promyelocytic leukemia, blocks the recruitment of HDACs to the transcriptional regulatory complex with RARα and causes tumor cell differentiation.[56,57]

Given the vast biologic effects of HDAC inhibition, one might expect HDAC inhibitors to have a narrow therapeutic window. However, data suggest that transformed cells are more sensitive to HDAC inhibitor-induced apoptosis than are normal cells. CTCL cells undergo higher rates of apoptosis than normal lymphocytes in response to HDAC inhibitor treatment.[58] Similarly, transformed human fibroblasts have a decreased growth rate and lower viability than normal fibroblasts when these cell types are grown in the presence of HDAC inhibitors.[59] These differences in sensitivity may be due to addiction of tumor cells to certain cellular pathways, concomitant genetic defects, or an inability of transformed cells to upregulate rescue pathways after a toxic insult.[60]

Unexpected, and perhaps contradictory, results reinforce the complexity of the cellular pathways influenced by HDACs. In vitro and in vivo, HDAC inhibitors cause cell cycle arrest and differentiation of many tumor types, including breast cancer.[61] However, in a study of invasive breast cancer, increased HDAC1 and 3 expression was paradoxically correlated with improved disease-free survival.[62] In patients with non–small-cell lung cancer (NSCLC), lower expression of class II HDACs was associated with a poor prognosis.[63] These contradictions underscore the caveats of predicting the effects of inhibiting a cellular pathway controlled by multiple isoenzymes which have complex tissue-specific and tumor-specific expression patterns.

HDAC INHIBITORS

HDAC inhibitors have attracted interest because of their ability to induce differentiation of malignant cells in culture.[64] The first of these was hexamethylene bisacetamide, and its more potent analogs of the so-called hybrid polar class. A related fungal product, trichostatin A, displayed similar differentiating effects in vitro. The activity of these compounds, all derivatives of hydroxamic acid, prompted the synthesis of vorinostat (suberoylanilide hydroxamic acid; Fig 2).[64,65]

To date, more than 15 HDAC inhibitors have been tested in preclinical and early clinical studies. The common mechanism of action of these drugs is to bind a critical Zn^{2+} ion required for catalytic function of the HDAC enzyme.[66] The detailed chemistry and development of these drugs have been reviewed.[17,67] The important clinical implication is that although these compounds were selected for their ability to inhibit histone deacetylation, they have widely varying potency and HDAC isoenzyme specificity, and variable effects on acetylation of nonhistone substrates (Table 2).[68] The various inhibitors

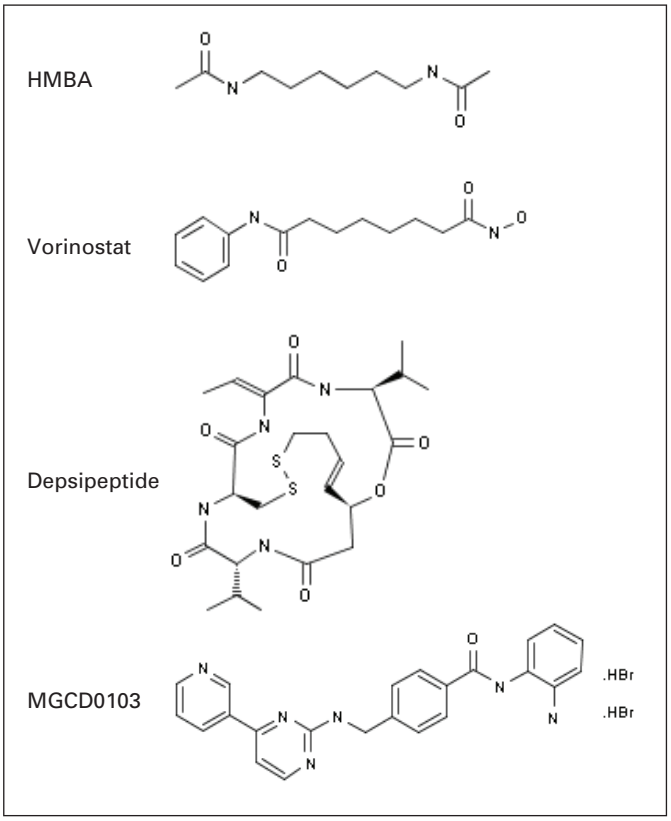

Fig 2. Chemical structure of selected histone deacetylase inhibitors. HMBA, hexamethylene bisacetamide.

Table 2. Selected HDAC Inhibitors in Clinical Use or Development

Compound by Class	Manufacturer	HDAC Class Specificity	In Vitro Potency*
Hydroxamic acid			
Vorinostat (Zolinza), suberoylanilide hydroxamic acid (SAHA)	Merck, Whitehouse Station, NJ	I, II, IV	μM
Trichostatin A (TSA)		I, II, IV	μM
LAQ824	Novartis, Basel, Switzerland	I, II, IV	nM
Panobinostat (LBH589)	Novartis	I, II, IV	nM
Belinostat (PXD101)	CuraGen, Branford, CT	I, II, IV	μM
ITF2357	Italfarmaco SpA, Cinisello Balsamo, Italy	I, II, IV	μM
Cyclic tetrapeptide			
Depsipeptide (romidepsin, FK228)	Gloucester Pharmaceuticals, Cambridge, MA	I (particularly HDAC1, HDAC2)	nM
Benzamide			
Entinostat (SNDX-275/MS-275)	Syndax Pharmaceuticals, Waltham, MA	I (particularly HDAC1, HDAC2, HDAC3)	μM
MGCD0103	Celgene, Summit, NJ; MethylGene, Montreal, Quebec	I	nM
Short-chain aliphatic acids			
Valproic acid		I, IIa	mM
Phenyl butyrate		I, IIa	mM
AN-9, pivanex	Titan Pharmaceutical, San Francisco, CA	NA	μM

Abbreviations: HDAC, histone deacetylase; NA, not available.
*Reported range of drug concentration necessary for in vitro inhibition of histone deacetylation in cell lines.

have nonoverlapping effects on transformed cells in vitro and would thus be expected to have differing efficacies, toxicities, and therapeutic uses in patients.

HDAC INHIBITOR PHARMACOLOGY

The only US Food and Drug Administration–approved HDAC inhibitor is vorinostat (Zolinza; Merck, Whitehouse Station, NJ).[64] The chemical class, HDAC isoenzyme specificity, and potency of vorinostat and selected inhibitors in clinical development, are listed in Table 2.[17,67] HDAC inhibitors have a relatively short half-life in plasma ($t_{1/2}$ approximately 2 hours for vorinostat,[69,70] 8 hours for depsipeptide,[71] 9 hours for MGCD0103[72]), and undergo hepatic metabolism. For depsipeptide, the principle enzyme responsible for metabolism is CYP3A4. The majority of the drug and its metabolites are eliminated via biliary and fecal routes; small amounts of parent and metabolites are detectable in the urine.[73] Similar pharmacokinetics are observed for vorinostat, which is metabolized principally via glucuronidation.[70] Interestingly, the HDAC inhibitors show pharmacodynamic effects well beyond the time of drug metabolism. For example, despite the short half-life of vorinostat in the blood, accumulation of acetylated histones in peripheral-blood cells continues up to 10 hours after an oral dose.[69]

RATIONALE FOR COMBINATION THERAPY

Multiple preclinical studies and clinical data support the use of HDAC inhibitors in combination with other cancer therapies.[67] Since HDAC inhibitors alter the balance in favor of proapoptotic pathways, they have been tested with conventional chemotherapeutic agents including platinums, taxanes, gemcitabine, fluorouracil, and epirubicin in solid tumors.[74] In addition, many of the earliest investigations of HDAC inhibitors were conducted in patients with myelodysplastic syndrome (MDS) and myeloid leukemia. These disorders exhibit abnormal recruitment of HDACs to nuclear protein complexes and have common recurring histone modifications.[75] These observations have formed the basis for combining HDAC inhibitors with the DNA methyltransferase inhibitor 5-azacytidine in MDS/acute myeloid leukemia (AML), or the differentiating agent all-*trans*-retinoic acid, in acute promyelocytic leukemia.[76,77] Finally, HDAC inhibitors enhance tumor cell radiosensitivity and are being tested with ionizing radiation in solid tumors.[78]

An important emerging target for HDAC inhibitors lies in the cellular mechanisms for handling misfolded proteins, which are degraded by the proteasome. Disruption of the proteasome system with bortezomib increases endoplasmic reticulum stress and apoptosis in multiple myeloma cells.[79] However, an alternative pathway, the aggresome, also participates in the disposal of ubiquitinated misfolded proteins.[80] This pathway is upregulated in the setting of proteasome inhibition and is dependent on the cytoplasmically localized HDAC6.[81] Inhibition of HDAC6 via short hairpin RNA, by the HDAC6-specific inhibitor tubacin, or by the pan-HDAC inhibitors vorinostat or LBH589, all resulted in synergistic apoptosis when combined with bortezomib.[82,83] These effects are also observed in nonmyeloma cell lines, suggesting a more generalizable target.[84] In addition to the proteasome and the aggresome, the cytoplasmic HSP system is also influenced by HDAC6, through deacetylation of lysines on HSP90 (Fig 1).[85] HDAC inhibition results in loss of HSP90 chaperone function and enhanced degradation of BCR-ABL, human epidermal growth factor receptor 2/*neu*, and FLT3; these data suggest potential synergy of HDAC inhibitors with imatinib, traztuzumab, or FLT3 inhibitors in cancers driven by amplified or mutated tyrosine kinases.[47]

HDAC INHIBITOR RESISTANCE

HDAC inhibitor resistance has been examined in vitro to further our understanding of HDAC biology, and to suggest strategies for rational

combination therapy. A mutation in HDAC2 was found in cell lines resistant to trichostatin A, and the same mutation was found in a subset of primary human tumor samples.[86] Other proposed mechanisms of HDAC inhibitor resistance include upregulation of cellular antioxidant pathways, increased expression of the antiapoptotic protein Bcl-2 and the stress-responsive transcription factor NF-κB, and use of alternative gene silencing pathways such as DNA methylation.[87] Finally, the unfolded protein response pathway is implicated in HDAC inhibitor resistance. An AML cell line resistant to growth inhibition induced by treatment with the hydroxamate class of drugs demonstrated hyperacetylation of HSP90 at baseline, but was then sensitive to treatment with 17-AAG, a geldanamycin derivative and HSP90 inhibitor.[88] 17-AAG synergizes with tubacin, an inhibitor of HDAC6, or with short interfering RNA against HDAC6, in killing primary leukemia cells.[89] Strategies to avoid resistance to HDAC inhibitors may employ combination therapies simultaneously targeting both HDACs and DNA methylation, or HDACs and HSP90.

CLINICAL TRIALS

A summary of selected HDAC inhibitor trials is shown in Table 3.[90-101] Due to space limitations, we discuss data on three agents of different classes with evidence of anticancer activity in phase II trials.

VORINOSTAT IN CTCL

Dose finding phase I trials of vorinostat were performed with both intravenous and oral formulations, in patients with advanced solid tumors and hematologic malignancies.[69,102] The maximum tolerated dose (MTD) of the oral formulation was 400 mg/d for continuous dosing. Dose-limiting toxicities (DLTs) were myelosuppression, fatigue, diarrhea, anorexia, and dehydration. Acetylated histones accumulated in peripheral blood mononuclear cells after therapy. Six of 73 patients had partial responses (PR), including one 17-month complete response (CR) in a patient with diffuse large B-cell lymphoma (DLBCL).

Advanced CTCL, a disease of malignant T-cell aggregates in cutaneous plaques and lymph nodes, is generally treated with the retinoid bexarotene, with the immunotoxin denileukin diftitox, or with systemic cytotoxics. Two phase II trials led to US Food and Drug Administration approval of vorinostat in CTCL.[9] A multicenter phase IIB trial enrolled 74 patients with progressive, persistent, or recurrent CTCL, who had received at least two prior therapies, including bexarotene.[92] The patients received vorinostat 400 mg orally daily as a single agent. The overall response rate (ORR) was 29.7%, with median duration of response of 6.1 months and median time to progression of 9.8 months (among stage IIB and higher responders). A phase II trial with a similar patient population found comparable results.[93] In 13 patients who received 400 mg/d, the ORR was 31%, while 24.2% responded among the entire study population of 33 patients who received varying doses of vorinostat. The median duration of response and time to progression were 15.1 and 30.2 weeks, respectively. Considering all patients in both phase II studies treated with 400 mg/d of vorinostat, the most common adverse events were diarrhea, fatigue, and nausea. Thrombocytopenia occurred in 26%, anemia in 14%. Grade 3 to 4 adverse events occurred in fewer than 5% of patients, and included thrombocytopenia, pulmonary embolism, fatigue, and nausea. Notably, no serious cardiovascular events were observed. The larger multicenter trial was recently updated in abstract form. Six of 74 patients remained on vorinostat for 2 years or longer with continued clinical effect (one CR, four PR, one stable disease), and minimal toxicity.[103]

OTHER VORINOSTAT TRIALS

In the limited number of trials reported, vorinostat has modest activity against solid tumors. A phase II study enrolled 27 women with

Table 3. Selected Clinical Trials of HDAC Inhibitors in Cancer

HDAC Inhibitor	Study/Population	Outcome
Vorinostat[90]	Phase I, AML or advanced hematologic malignancy, n = 41	2 CR, 2 CR with incomplete count recovery, 3 hematologic responses
Vorinostat (with carboplatin and paclitaxel)[91]	Phase I, advanced solid tumors, n = 25	11 PR (10 NSCLC, 1 head and neck); 7 SD
Vorinostat[92]	Phase IIb, CTCL, n = 74	1 CR, 21 PR; ORR, 29.7%; DOR, 6.1 months; TTP, 9.8 months
Vorinostat[93]	Phase II, CTCL, n = 33	8 PR; ORR, 24.2%; DOR, 15.1 weeks; TTP, 30.2 weeks
Vorinostat[94]	Phase II, relapsed DLBCL, n = 18	1 CR, 1 SD
Vorinostat[95]	Phase II, relapsed indolent NHL, n = 17	4 CR, 2 PR, 4 SD
Depsipeptide[96,97]	Phase I/II, CTCL or PTCL, n = 53	3 CR, 2 PR (of 27 CTCL or PTCL); 3 CR, 8 PR (of 36 PTCL)
Depsipeptide[98]	Phase II, hormone refractory prostate cancer, n = 31	1 PR, 6 SD; 7% PSA response rate
Depsipeptide[99]	Phase II, metastatic renal cell cancer, n = 29	1 CR, 1 PR
MGCD0103[72]	Phase I, AML or myelodysplastic syndrome, n = 29	3 CR
MGCD0103[100]	Phase II, relapsed/refractory Hodgkin's lymphoma, n = 21	2 CR, 6 PR; ORR, 38%
MGCD0103[101]	Phase II, relapsed/refractory DLBCL, n = 17	1 CR, 3 PR; ORR, 24%

Abbreviations: HDAC, histone deacetylase; CR, complete response; PR, partial response; NSCLC, non–small-cell lung cancer; ORR, overall response rate; CTCL, cutaneous T-cell lymphoma; PTCL, peripheral T-cell lymphoma; n, number of patients in trial; SD, stable disease; DOR, duration of response; TTP, time to progression; DLBCL, diffuse large B-cell lymphoma; NHL, non-Hodgkin's lymphoma; AML, acute myeloid leukemia.

platinum-resistant epithelial ovarian cancer or primary peritoneal carcinoma for treatment with vorinostat 400 mg/d. Two women were progression free at 6 months, and one had a PR.[104] A small phase II trial of single-agent vorinostat in metastatic head and neck cancer yielded no confirmed PRs or CRs.[105] An encouraging phase I study added vorinostat on a dose escalation schedule to carboplatin and paclitaxel in advanced solid malignancies.[91] Eleven of 25 patients (10 of 19 patients with NSCLC and one of four with head and neck cancer) achieved a PR. Vorinostat metabolism was delayed when combined with paclitaxel/carboplatin, but paclitaxel pharmacokinetics were unaffected. These data have led to an ongoing phase II National Cancer Institute–sponsored trial of vorinostat with paclitaxel/carboplatin in NSCLC. Early results of other phase II studies of single agent vorinostat in solid tumors have been presented, with isolated responses in NSCLC,[106] glioblastoma multiforme,[107] and breast cancer.[108,109]

HDAC inhibitors are also showing clinical promise in B-cell lymphomas; vorinostat trials have only been published in abstract format thus far. A phase II study of oral vorinostat in relapsed DLBCL showed a CR in one of 18 patients for more than 468 days and stable disease in one patient for 301 days.[94] A second phase II trial was performed in 17 patients with relapsed indolent non-Hodgkin's lymphoma treated with vorinostat 200 mg twice daily for 14 days of a 21-day cycle. Four patients achieved a CR, two had PRs, and four patients had stable disease.[95]

Phase I data also demonstrated activity of oral vorinostat as single agent therapy in AML. A dose escalation study using oral vorinostat in 41 total patients enrolled 31 with AML, three with MDS, four with chronic lymphocytic leukemia, two with acute lymphoblastic leukemia, and one with chronic myeloid leukemia. The MTD on two different dosing schedules was 200 mg twice daily or 250 mg three times daily, each given for 14 days of a 21-day cycle. DLTs were fatigue, nausea, vomiting, and diarrhea. Seven patients with AML had hematologic responses, including two CRs and two CRs with incomplete count recovery.[90]

DEPSIPEPTIDE

The cyclic tetrapeptide depsipeptide had clinical efficacy when given by intravenous infusion in a case series of four patients with CTCL or peripheral T-cell lymphoma (PTCL). Three patients with CTCL had a PR, and one patient with PTCL achieved a CR after 6 months of therapy.[96] In a parallel phase I study that did not include those four patients, depsipeptide had a favorable safety profile, and the MTD was 17.8 mg/m^2 given on days 1 and 5 of a 21-day cycle. The DLTs included fatigue, nausea, vomiting, thrombocytopenia, and atrial fibrillation.[71] The same authors have presented interim data of a phase II trial of depsipeptide in CTCL or PTCL, with an ORR of 37% (three CRs and seven PRs in 27 patients).[97] Several phase I trials have found little to no clinical benefit of single-agent depsipeptide in refractory neoplasms including AML/MDS, CLL, lung cancer, and renal cell cancer.[99,110-113] A phase II trial of single-agent depsipeptide in 31 patients with hormone refractory prostate cancer showed a short-term disease control rate of 14% (radiographic PR or disease stabilization) and a prostate-specific antigen response rate of 7%.[98] Despite modest clinical efficacy, the drug was relatively well tolerated and thus these data suggest that combination clinical trials of depsipeptide with cytotoxic chemotherapy, or with other targeted agents, may be warranted.

MGCD0103

The benzamide MGCD0103 is an orally bioavailable HDAC inhibitor with activity in hematologic malignancies, including myeloid leukemia and lymphoma. Phase I data demonstrated a favorable safety profile and showed activity as a single agent leading to a bone marrow CR in three of 29 patients with AML. The MTD was 60 mg/m^2 administered orally three times weekly, with DLTs of fatigue, nausea, vomiting, and diarrhea.[72] In interim analysis of an ongoing phase II trial of MGCD0103 in relapsed or refractory Hodgkin's lymphoma, 38% of patients responded, with median time to response of 8 weeks. Six of 21 patients had a PR, and two had a CR with ongoing progression-free survival of 270 and 420 days respectively, at time of reporting.[100] Similar encouraging results were seen with MGCD0103 in a phase II trial in relapsed or refractory DLBCL, where a response rate of 24% was reported in a small cohort. Of 17 patients, one CR and three PRs were achieved, with duration of response ranging from 112 to 336 days.[101]

OTHER INHIBITORS

Several other HDAC inhibitors have shown promise in early phase I or small phase II trials. The hydroxamate panobinostat (LBH589)[114,115] and the benzamide entinostat (SNDX-275)[116-119] both have attractive preclinical and phase I safety and efficacy profiles, with evidence of activity in CTCL and AML. Like vorinostat, panobinostat and entinostat are active against transformed cells in culture, and trials are ongoing in relapsed and refractory lymphoid malignancies, myeloid leukemia, and solid tumors.

Early clinical data suggest utility of valproic acid as an HDAC inhibitor, in combination with hypomethylating agents 5-azacitidine or 5-aza-2′-deoxycytidine, or with the differentiating agent retinoic acid, in AML or advanced MDS.[120,121] Although generally well-tolerated, valproic acid may be eclipsed by more potent and specific HDAC inhibitors. In addition, numerous phase I trials and preclinical data beyond the scope of this review justify further clinical studies of HDAC inhibitors alone or in combination with cytotoxic or targeted drugs in cancer (a more comprehensive list of clinical trials data has been recently reviewed[74]).

TOXICITY

In phase I and II trials, the safety profile of HDAC inhibitors has been favorable, especially in comparison to traditional cytotoxic chemotherapy. Combination therapy with chemotherapeutic drugs has not required substantial dose modification of either the HDAC inhibitor or the cytotoxic drug(s). The most frequent toxicities, common to most HDAC inhibitors tested, are fatigue, nausea, and diarrhea. Myelosuppression is relatively mild, with thrombocytopenia predominating over anemia or neutropenia.[9]

The most worrisome adverse effect has been cardiac toxicity, including ventricular arrhythmia. This toxicity may be a class effect of

the HDAC inhibitors, and has been proposed to occur through interaction with the HERG K^+ channel.[122,123] A phase II study of depsipeptide in 15 patients with metastatic neuroendocrine tumors was halted early after several cardiac events occurred. One patient may have died from a fatal ventricular arrhythmia, while two had asymptomatic nonsustained ventricular tachycardia and three developed a prolonged QTc interval.[124] A systematic study of cardiac function was performed in a subset of patients enrolled in a phase II trial of depsipeptide in CTCL. Transient ECG changes (T wave flattening, ST segment depression) occurred in more than half of patients after intravenous infusion, and almost all patients had a small increase in the QTc interval (median increase 14 milliseconds).[125] Phase II and III studies of this agent have proceeded without interruption after this regulatory review.

Serious cardiac toxicity has not been reported with vorinostat. In a phase II trial in CTCL, 15 of 74 patients had grade 1 to 2 electrocardiographic changes, including three with QTc prolongation.[92] The hydroxamate LBH589 and its structural predecessor LAQ824 both prolong the QTc interval.[126,127] Although one patient on intravenous LAQ824 developed 10 seconds of torsades de pointes, most patients had asymptomatic prolongation of the QTc by fewer than 20 milliseconds. Studies of both depsipeptide and LAQ824 revealed no long-term changes in echocardiographic parameters.[125,127] However, because of toxicity concerns, patients with significant heart disease, baseline prolonged QTc interval, or those who need medications which prolong the QTc, have been excluded from HDAC inhibitor trials. Cardiac toxicity may be associated with inhibitor potency, as there is less QTc prolongation with vorinostat than the more potent hydroxymate LBH589. Phase I trials with entinostat have not revealed significant evidence of QTc prolongation, suggesting that separation of efficacy and cardiac toxicity may be possible.[116,117,119]

BIOMARKERS

Molecular analysis of tumor samples would ideally discriminate which patients would benefit from HDAC inhibitor therapy. Knockdown of HDAC1, but not HDAC2 or HDAC3, conferred partial resistance to belinostat-induced cell death in a human cervical cancer cell line.[128] Although these data are provocative and suggest that high HDAC1 levels may be associated with sensitivity to inhibitor treatment, further study will be needed to determine if a tumor-specific HDAC isoenzyme profile predicts response to individual HDAC inhibitors. Non-HDAC gene expression patterns may also predict response to treatment. Molecular profiling of NSCLC cell lines treated with trichostatin A or vorinostat showed that a nine gene RNA expression signature predicted sensitivity to HDAC inhibitor-induced apoptosis.[129] A retrospective analysis of pretreatment CTCL skin biopsies found that high nuclear STAT1 and phospho-STAT3 staining in lymphoma cells correlated with lack of clinical response to vorinostat.[130] Clinical studies have not yet used such biomarkers to select patients, or to predict response to HDAC inhibitor treatment.

SUMMARY

HDAC inhibitors have shown promise in therapy for human lymphoid cancers in early clinical trials. Vorinostat is approved for the treatment of relapsed or refractory CTCL, and other HDAC inhibitors, particularly depsipeptide, appear to have clinical benefit in this disease, while MGCD0103 produced multiple responses in lymphoma and AML. In general, HDAC inhibitors are well tolerated with minimal adverse effects, although cardiotoxicity, particularly arrhythmia, may be a class toxicity that needs to be evaluated further in larger populations of treated patients and warrants caution in using HDAC inhibitors in patients with underlying heart disease.

An important consideration moving forward is the significant diversity in the cellular pathways affected by HDACs. In addition to deacetylation of histones, these enzymes affect the acetylation status of many other nuclear and cytoplasmic proteins, including the important chaperone HSP90. Inhibitors of HDACs appear to have global effects. HDAC inhibition may thus not be a targeted therapy in comparison to kinase inhibitors or monoclonal antibodies, as it has broader, and at this point incompletely understood, effects on a wide array of cellular proteins.

There are several HDAC inhibitor drugs available or in clinical development, differing in potency and enzyme specificity. Given the protean actions of HDACs and the differences in the effects of individual HDAC inhibitors, it may be incorrect to make generalizations based on results with specific drugs in laboratory testing or clinical trials. Several important questions remain. Which HDAC enzymes are most critical in maintaining a neoplastic phenotype? Will the most effective drugs narrowly target one class or a single HDAC, or will the less specific HDAC inhibitors succeed by influencing multiple cellular pathways simultaneously? Are the adverse effects, particularly cardiac, linked to inhibition of only certain HDAC enzymes, and could more isoenzyme-specific inhibitors have an improved therapeutic window? Preclinical investigation by targeted knockdown of individual HDAC isoenzymes, or by development of more isoenzyme-specific inhibitors for clinical use, may be required to elucidate these subtle biologic differences.

Despite the yet unanswered biologic questions, and while these drugs may not act as directly targeted therapies, they appear to alter the balance of a tumor cell such that it is more prone to differentiation, growth arrest, and apoptosis. Given the early success of HDAC inhibitors in several cancers, we anticipate further benefits of this new class of drugs, both as single agents and in combination therapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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